Voltage-gated Na Channels I

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NavAb Structure as a Template to Rationalize Experimental Data on Nav1.4 Block by Mu-Conotoxins

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In crystallographic structures of NavAb and other bacterial sodium channels the P-loop domain contains P2 helices, which are absent in potassium channels. Here we asked whether these structures can advance understanding of experimental data accumulated for eukaryotic sodium channels. Using published experimental data on interactions of mu-conotoxins with Nav1.X channels, we docked GIIIA, PIIIA and KIIIA in the NavAb-based model of Nav1.4. Docking of GIIIA was based on specific channel-toxin contacts described in published experimental studies. Importantly, these specific contacts were readily reproduced in our computations with Monte Carlo-energy minimizations without modifications of the template backbone geometry. Computed energies of specific interactions correlated with experimental estimations. Predicted orientation of the GIIIA was used to dock PIIIA and KIIIA. The obtained toxin-channel complexes are consistent with mutational data and voltage-dependence of toxin action. Particularly, tetrodotoxin can pass between Nav1.4 and the channel-bound KIIIA to reach its binding site in the selectivity filter. KIIIA and some GIIIA and PIIIA mutants are known to incompletely block the current. To understand this phenomenon, we Monte Carlo-minimized the energy of toxin-channel complexes from many starting points with randomly placed sodium ions and superimposed low-energy structures to visualize constellations of the ions. Uninterrupted pathways of sodium ions between the extracellular space and the selectivity filter where seen only when at least one outer carboxylate was not salt-bridged to the toxin. A good correlation was found between the modeling results and experimental data on complete and incomplete channel block by the native and mutant toxins. Thus, the NavAb structure advances understanding of permeation and block of eukaryotic sodium channels. Our study suggests similar folding of the outer-pore region in eukaryotic and prokaryotic sodium channels. Supported by RFBR-13-04-00724 to DBT and NSERC to BSZ.

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Effects of the Protonation States of the EEEE Motif of a Bacterial Na^+-Channel on Conduction and Pore Structure

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A distinctive feature of prokaryotic Na^+-channels is the presence of four glutamate residues in their selectivity filter. How the structure of the selectivity filter and the free-energy profile of a permeating Na^+ ion are altered by the protonation state of Glu177 residues in the NaVAb channel have been the focus of this study. It was found that protonation of a single glutamate residue is sufficient to modify the behavior of the selectivity filter. Molecular dynamics simulations reveal that the side chain of glutamates can adopt at least two orientations depending on the protonation state. The likelihood of a conformation where Glu177 points toward the inside of the selectivity filter is correlated with an energy barrier for ion translocation between the intracellular and extracellular sides of the channel. This energetic barrier precludes Na^+ ions to permeate the selectivity filter of prokaryotic Na^+-channels with protonated glutamates by a mechanism described for channels with four charged glutamates.

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Structure of a Prokaryotic Sodium Channel Pore Reveals Essential Gating Elements and an Outer Ion Binding Site Common to Eukaryotic Channels

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Voltage-gated sodium channels (NaVs) are central elements of cellular excitation. Notwithstanding advances from recent bacterial NaV (BacNaV) structures, key questions about gating and ion selectivity remain. Here, we present a closed conformation of NaVAe1p, a pore only BacNaV derived from NaVAe1, a BacNaV from the arsenite oxidizer Alkalilimnicola ehrlichei found in Mono Lake, California, that provides insight into both fundamental properties. The structure reveals a pore domain in which the pore-lining S6 helix connects to a helical cytoplasmic tail. Electrophysiological studies of full-length BacNaVs show that two elements defined by the NaVAe1p structure, an S6 activation gate position and the cytoplasmic tail 'neck', are central to BacNaV gating. The structure also reveals the selectivity filter ion entry site, termed the 'outer ion' site. Comparison with mammalian voltage gated calcium channel (CaV) selectivity filters, together with functional studies shows that this site forms a previously unknown determinant of CaV high affinity calcium binding. Our findings underscore commonalities between BacNaVs and eukaryotic voltage gated channels and provide a framework for understanding gating and ion permeation in this superfamily.

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Uncovering the Links Between Conformational Flexibility and Function for a Bacterial Voltage-Gated Sodium Channel

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Voltage-gated sodium channels play essential roles in electrical signaling in the nervous system and are key pharmacological targets for a range of disorders. We have carried out fully-atomistic simulations on the multi-microsecond timescale to investigate sodium channel function. These simulations have produced complete unbiased free energy maps that reveal complex multi-ion conduction mechanisms for sodium (as well as potassium and calcium ions). They have also uncovered a surprising level of conformational flexibility of the channel, including concerted movements of the selectivity filter's EEEE ring sequence side chains, and significant changes in pore domain structure as a function of glutamate protonation states. We have observed asymmetrical rearrangements of the activation gate, resembling previously proposed inactivated structures, as well as helix bending involving residues critical for slow inactivation. We report how these structural changes regulate access to lipid-facing fenestrations and the binding of the local anesthetic and anticonvulsant drugs phenytoin and benzocaine, providing new insight into the molecular mechanisms of sodium channel inhibition.

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Structural Modeling of Toxin Interactions with the Human Voltage-Gated Sodium Channel Pore

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Voltage-gated sodium (Nav) channel subtypes are targets for the development of novel analgesics. We are constraining structural models of human Nav subtypes, as the lack of high-resolution structures of channels makes rational drug design challenging. The human Nav1.4 channel is associated with paralysis and human Nav1.7 channel plays an important role in pain signaling. We used the bacterial Nav channel NavRh (pdb id: 4DXW) as a template to generate homology/de novo models of human Nav1.4 and Nav1.7 channel pore-forming domains. Due to significant sequence differences between human and bacterial Nav channels in the P2 helix region of the selectivity filter, we predicted the structure of these regions de novo using Rosetta loop modeling methods. We simulated interactions of human Nav1.4 and Nav1.7 channels with pore blocking toxins - tetrodotoxin, saxitoxin, and μ-conotoxin KIIIA - using Rosetta methods and molecular dynamics in an all-atom explicit membrane environment. Virtual alanine scans of key residues forming toxin receptor sites within human Nav1.4 and Nav1.7 channel pore-forming domains showed good correlation with available experimental data. High-resolution structural models of the human Nav channels provide critical starting points for design of novel analgesics.

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Finding the Route of Entry and Binding Site of Local Anaesthetics in Bacterial Voltage Gated Sodium Channels Using Molecular Dynamics Simulation

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Voltage-gated sodium channels are proteins in cell membranes that initiate action potentials in neurons. Dysfunctional sodium channels are implicated in diseases such as chronic pain, epilepsy and cardiac arrhythmia, which are often treated by sodium channel blockers such as the family of local anaesthetics. These blockers can have adverse side-effects by blocking healthy channel subtypes, so researchers have strived to understand their mechanism of action to help design new, subtype-specific drugs. While there is no atomic